IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicants:	Johnson et al.
Appl. No.:	08/458,019
Conf. No.:	2660
Filed:	1 June 1995
Title:	PROCESSES FOR IN VIVO PRODUCTION OF ASTAXANTHIN AND PHAFFIA RHODOZYMA YEAST OF ENHANCED ASTAXANTHIN CONTENT
Art Unit:	1651
Examiner:	LILLING, Herbert J.
Docket No.:	116588-002

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD DECLARATION OF STEPHEN HIU

UNDER 37 C.F.R. § 1.132

Sir:

I, Stephen Hiu, hereby declare and state:

My qualifications and current responsibilities are set forth in my First and Second Declarations.

I reviewed the Office Action of 4 October 2006 as well as the Johnson et al. 1980 publication, and the Johnson & Lewis 1979 publication and Murillo et al. publication referred to by the Examiner in the rejection.

The Examiner cited Johnson's 1980 publication where it is stated that "The titer of astaxanthin of the wild-type strain of *P. rhodozyma* varies from 30-800 ug/g yeast, depending on the growth conditions (Johnson and Lewis, 1979)." A careful examination of the Johnson and Lewis 1979 publication will show that nowhere in this document is an astaxanthin concentration of 800 µg/g yeast mentioned. Indeed, the highest indicated astaxanthin concentration is 652 µg/g yeast (Table 3, p.178) when grown on cellobiose.

With respect to the Murillo citation, I offer the following comments. At the time of these publications, *Phaffia* was known to be an asporagenous yeast strain that did not have sexual phase. In contrast, *Phycomyces* is a fungus that reproduces via a sexual phase. *Phycomyces* produce spores that contain multiple nuclei. These spores can come together to form multinucleate heterokaryons.

A person skilled in the art would not have been able to apply the procedures of Murillo to *Phaffia*. The reason is that the procedures of Murillo et al. apply to *Phycomyces* which has a sexual phase and a different means of propagation that cannot be applied to *Phaffia*. *Phycomyces* produce spores which can randomly assort and combine so that the likelihood of having complementing genes is very high, whereas *Phaffia* propagate by budding. Also, unlike *Phaffia, Phycomyces* produce multinucleate heterokaryons which greatly increase the likelihood of obtaining a cell which has complementing genes to overproduce to desired carotenoid. On the other hand, because *Phaffia* is a unicellular organism that contains only a single nucleus, the likelihood of obtaining a mutant that carries one or more mutations that yield a strain that overproduces astaxanthin is much less frequent and unpredictable.

It was not until 1995 that terminal basidiospores were first observed in *Phaffia* (Golubev, 1995, Perfect State of *Rhodomyces dendrorhous* (*Phaffia rhodozyma*) Yeast 11:101-110) when grown under very specific environmental conditions and on specialized agar media containing polyols. Therefore, at the time of the Johnson and Murillo publications, a person skilled in the art could not have applied the methods taught by Murillo.

Following the direction of the above application, we have subsequently produced additional strains of *Phaffia* that yield high levels of astaxanthin when grown and measured as described in Claim 25. (Table 1 below). Furthermore, some of these strains have now been commercialized to yield a dried *Phaffia* product containing 10,000 µg astaxanthin/g yeast .

Table 1:

Strain Name	ppm
GCH001	6254
GCH002	7132
CI9	7273
BNY20	5973
C12	7766

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Further Declarant sayeth not.

Date: 3/19/07

Stephen Hiu